APPENDIX A

(http://pathmicro.med.sc.edu/pcr/realtime-home.htm

"Thus, as we have emphasized, quantitation of the amount of cDNA in the original sample must be done where the amplification is exponential and, as we saw above, this is at the very beginning of the upturn of the curve and not in what **appears** to the linear region of the curve.

In real time PCR, we measure the **cycle number at which the increase in fluorescence (and therefore cDNA) is exponential**. This is shown by the orange horizontal line in the figure (known as the threshold) and is set by the user. The point at which the fluorescence crosses the threshold is called the **Ct**.

It should also be noted that samples that differ by a factor of 2 in the original concentration of cDNA (derived from mRNA) would be expected to be 1 cycle apart. Thus samples that differ by a factor of 10 (as in our dilution series) would be ~3.3 cycles apart."

http://www.dorak.info/genetics/glosrt.html
GLOSSARY OF REAL-TIME PCR TERMS
M.Tevfik Dorak

"**C_t (threshold cycle)**: Threshold cycle reflects the cycle number at which the fluorescence generated within a reaction crosses the threshold. It is inversely correlated to the logarithm of the initial copy number. The C_t value assigned to a particular well thus reflects the point during the reaction at which a **sufficient number of amplicons have accumulated.** Also called crossing point **(C_p)** in LightCycler terminology. "

> **Refers to Applied Biosystems publication: Understanding C_t Value which is found at the URL:**
> http://www3.appliedbiosystems.com/cms/groups/mcb_marketing/documents/generaldocuments/cms_053906.pdf
> and appears as Appendix B